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EXPERIMENTS ON THE ORIGINS OF OPTICAL ACTIVITY

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Two recent reports in the literature have struck us as novel and potentially important enough to require further experimental substantiation. These involve the claims that kaolin a) adsorbs L-phenylalanine from 10^{-3} $\underline{M}$ aqueous solution more extensively than D-phenylalanine at pH 6 (the reverse being true at pH 2)(Jackson 1971a, 1971b) and b) induces the polymerization of L-aspartic acid faster than D-aspartic acid in 10^{-2} $\underline{M}$ solution at 90° (Degens et al., 1970; Jackson, 1971a). The potential significance of these observations for the origin of optically active compounds in nature, as well as the absence of any chiral crystal lattice in kaolin which might theoretically induce such results, have prompted us to duplicate these experiments using more sensitive and varied experimental techniques.

We first attempted to observe the asymmetric adsorption of phenylalanine enantiomers by kaolin under the literature conditions (Bonner and Flores, 1973). D,L-Phenylalanine in 10^{-3} $\underline{M}$ pH 6 solution was stirred with kaolin, then centrifuged. The supernatant (Fraction 6-1; Table I) was quantitatively analyzed for phenylalanine using an amino acid analyzer, the kaolin residue was extracted with 4 $\underline{M}$ NH_4OH, then was centrifuged, and the second supernatant (Fraction 6-2) was similarly analyzed. An analogous adsorption experiment was conducted using pH 2 HCl solvent, again providing two fractions (2-1; 2-2; Table I). The "adsorption" by kaolin and the total recovery of phenylalanine between the two fractions of each experiment is shown in Table I. The pH 6 adsorption values are considerably lower than those previously reported (Jackson, 1971a, 1971b).

Table I. Retention of D,L-Phenylalanine by Kaolin in Aqueous Solution

Fraction	pH	% Phenylalanine Retained with Supernatant[a]	Kaolin[b]	Total Recovery, %
6-1	6	93.2	6.8	102
6-2	6	8.8	0	
2-1	2	68.6	31.4	81.6
2-2	2	13.0	18.4	

a. By amino acid analyzer analysis;

b. By difference.

The solute from each fraction in Table I was recovered and examined in a spectropolarimeter to determine its optical rotatory dispersion curve in the 280-625 nm region. If asymmetric adsorption of D,L-phenylalanine had indeed occurred, it was hoped that this could be detected by measurable optical activity at those wavelengths corresponding to maximum (or minimum) specific rotations of authentic samples of L-phenylalanine at pH 6 and its hydrochloride at pH 2. In none of the recovered samples, however, was an observed rotation in excess of +0.005° (from that of the solvent blank) noted at any wavelength used, suggesting little, if any, optical activity. Unfortunately, however, such ORD data could not be considered definitive, since the "predicted observed rotations" of our samples (based on Jackson's (1971a, 1971b) reported adsorption values and the known solute concentrations in our ORD solutions) were within or close to the observed rotation values which we found. We therefore sought alternative criteria for asymmetric adsorption in the four fractions in Table I.

The phenylalanine in each of the above ORD fractions was recovered by lyophilization and each sample was converted to a diastereomeric mixture of its N-trifluoroacetyl-(+)-2-butyl ester derivative (Pollock et al., 1965). These mixtures, along with similar D,L-phenylalanine derivative controls, were analyzed quantitatively for their D- and L-enantiomeric compositions by gas chromatography on a 150' × .02" capillary column (OV-225 phase). These analyses are summarized in Table II, where we see that each fraction has an enantiomeric composition identical within experimental error to that of the D,L-phenylalanine control. By contrast, Jackson's (1971a, 1971b) adsorption data would predict enantiomeric compositions for the recovered phenylalanine of 51.2% D- and 48.8%

L- for the pH 6 experiment, and 48.4% D- and 51.6% L- for the pH 2 experiment.

Table II. GC Analyses for Enantiomeric Composition of Phenylalanine Fractions Isolated After Kaolin Treatment

Fraction	% Phenylalanine Enantiomer	
	D	L
6-1	49.9	50.1
6-2	49.7	50.3
D,L-Control	49.8	50.2
2-1	50.2	49.8
2-2	50.0	50.0
D,L-Control	50.8	49.2

To confirm the absence of asymmetric adsorption of phenylalanine by kaolin we lastly resorted to thin layer chromatography. Two TLC plates were coated with kaolin as adsorbant, and samples of D-, L- and D,L-phenylalanine were spotted in quadruplicate on each. One plate was developed with water and one with pH 2 HCl, whereupon the plates were dried and the spots visualized with ninhydrin. Differences in adsorption of the two enantiomers by the kaolin adsorbant should, of course, show up as different

R_f values. All twelve spots on each plate, however, had R_f values on that plate identical within experimental error, indicating again no preferential adsorption of either phenylalanine enantiomer and no resolution of the racemic phenylalanine by kaolin.

We next investigated (Flores and Bonner, 1973) the reported (Degens _et al_., 1970; Jackson, 1971a, 1971b) asymmetric polymerization of aspartic acid by kaolin in aqueous suspension at 90°. 0.01 _M_ Solutions of D,L- (0.05 mmole) and L-aspartic acid (0.10 mmole) were treated with kaolin in sealed tubes for 8 days at 90°, duplicating literature conditions. The kaolin was filtered and washed with water (Fractions 1, Table III), then was reextracted thrice with 2 _M_ NH_4OH (Fractions 2) to remove any remaining adsorbed aspartic acid. The fractions were each treated with known quantities of D,L-threonine as an internal standard and were then analyzed each in triplicate for their Asp/Thre ratio on an amino acid analyzer, thus providing accurate assays of the aspartic acid content of each fraction. As summarized in Table III, we see that both for D,L- and L-aspartic acid the total recovery of unchanged material was essentially quantitative (> 95%), suggesting

Table III. Recovery of D,L- and L-Aspartic Acid after Treatment with Kaolin

Fraction	mMoles	% of Total	
D,L-1	.0485	97.0 ± 1.0	~ 98.2
D,L-2	.0006	1.2	
L-1	.0942	94.2 ± 3.2	~ 95.4
L-2	.0012	1.2	
L-1 (Hydrolyzed)	.0959	95.9 ± 2.8	

that only trivial -- if any -- polymerization had actually occurred. These data are in sharp contrast to Jackson's reports (1971a, 1971b), which maintain that L-aspartic acid is 40% and D-aspartic acid 15% polymerized under these conditions. To see if the slightly below quantitative recovery of L-aspartic acid in the L-1 fraction of Table III might actually be due to the presence of poly-aspartic acid, we subjected this fraction to 20-hour hydrolysis with refluxing 6 N HCl, then re-analyzed for aspartic acid using a threonine internal standard as before. As seen in Table III the aspartic acid content of Fraction L-1 was unchanged after hydrolysis, thus indicating again that little if any polymer was present.

For confirmation, we next looked for the asymmetric polymerization of aspartic acid by a completely independent technique, namely, examination of the D/L ratio in the residual aspartic acid recovered after treatment of D,L-aspartic acid with kaolin at 90° for 8 days. If, as reported, L-aspartic acid polymerizes faster than D-aspartic acid, the D/L ratio in the recovered sample should be >1, (D/L = 1.417 on the basis of Jackson's data). Our above results, of course, would predict that the D/L ratio should be 1.000.

We determined the desired D/L ratios by quantitatively converting the recovered kaolin-treated aspartic acid into diastereomeric mixtures of L-Leu-D-Asp and L-Leu-L-Asp after reaction with excess L-leucine N-carboxy anhydride (Manning and Moore, 1968). The diastereomers were readily and quantitatively separated on the amino acid analyzer, whereupon peak area measurements on the triplicately-run chromatograms allowed accurate determination of the desired D/L ratios. As seen in Table IV (No. 1) the recovered D,L-aspartic acid had a D/L ratio within experimental error of 1.000. This was unchanged after acid hydrolysis (No. 2), indicating no increase in either enantiomer due to acid hydrolysis of any polymer which might have been present.

Table IV. Enantiomeric Composition of Kaolin-Treated Aspartic Acid Samples before and after Acid Hydrolysis

No.	Sample	D-Asp/L-Asp	Enantiomeric Composition	
			%-D	%-L
1	D,L	1.008 ± .009	50.2	48.8
2	D,L (Hydrolyzed)	1.000 ± .003	50.0	50.0
3	L	0.455 ± .014	31.3	68.7
4	L (Hydrolyzed)	0.461 ± .006	31.5	68.5
5	(D,L-Standard)[a]	(1.000 ± .006)	(50.0)	(50.0)

a. Control, untreated with kaolin.

Since the action of kaolin on D- and L-aspartic acid solutions at 90° has been reported to cause racemization (Degens et al., 1970; Jackson, 1971a, 1971b), it was of course possible that the 1:1 D/L ratios for Nos. 1 and 2 in Table IV were due to racemization of both unpolymerized aspartic acid enantiomers during the kaolin treatment, rather than to the absence of asymmetric polymerization. To test this possibility we repeated the experiment with L-aspartic acid, analyzing the recovered product again for its D/L ratio. The product proved 31.3% converted into

D-aspartic acid (No. 3, Table IV), indicating extensive (62.6%) racemization by the kaolin treatment. Finally, the latter sample was hydrolyzed with HCl to see if this changed the enantiomeric ratio. The D/L ratio of No. 4 (Table IV), however, was identical to that of the unhydrolyzed sample No. 3. This clearly confirms that the L-aspartic acid had not polymerized appreciably, since if it had, acid hydrolysis of the polymer would have liberated additional L-aspartic acid, thus <u>decreasing</u> the D/L ratio in No. 4. All of our experimental data thus accord with the conclusions that a) there is no significant gross polymerization of aspartic acid in 0.01 <u>M</u> solution by kaolin during 8-day treatment at 90°, and b) there is likewise no selective polymerization polymerization of L- over D-aspartic acid under these same conditions. Since completing our studies we have learned that McCullough and Lemmon (1973) have attempted similar aspartic acid polymerization experiments, and have come to identical conclusions using analytical techniques completely different from ours.

In 1959 Vester and coworkers proposed a novel hypothesis for the natural origin of optically active compounds, based on the known violation of the parity principle during β-decay of certain radioactive isotopes (Wu <u>et al</u>., 1957). In this mechanism the predominantly "left-handed" longitudinally polarized electrons emitted during β-decay interact with matter to form circularly polarized γ- and X-ray Bremsstrahlung photons, which in turn may induce absolute asymmetric syntheses or degradations in appropriate organic molecules, thus yielding optically active products. Early attempts to demonstrate this mechanism experimentally (Vester _et al._, 1959; Ulbricht and Vester, 1962; Gary, 1968), however, have led to negative or ambiguous results, presumably due to the relatively weak β-sources employed and to the short exposure times. We have attempted to examine this mechanism experimentally using a considerably more powerful β-ray Bremsstrahlung source, namely, 61000 Curies of strontium-90 housed at Oak Ridge National Laboratory. After demonstrating that γ-radiation was in fact capable of causing degradation of amino acids in the solid state (Bonner, 1973), we placed samples of D-, L- and D,L-leucine, both as solids and as salts in solution, in the above ^{90}Sr source, and irradiated them for a total of 11,736 hours (1.34 years; total dose 4.18×10^8 rads). The recovered amino acid samples were converted to diastereomeric mixtures of their volatile N-trifluoroacetyl (+)-2-butyl esters (Pollock et al., 1965), then were assayed for percent degradation gas chromatographically, using the technique of enantiomeric markers (Bonner, 1973). The recovered D,L-leucine samples were also examined for enantiomeric inequality by optical rotatory dispersion measurements and again by gas chromatography of their N-trifluoroacetyl (+)-2-butyl esters. The results of these experiments are summarized in Tables V and VI.

Examination of Table V indicates that there is no preferential radiolysis of either enantiomer of the leucine salts in aqueous solution, even after total degradation has proceeded to the extent of almost 50%. Such an observation, while contrary to the report of Garay (1968), however, is what one would probably anticipate for radiolysis conducted in aqueous solution. Here the initial mechanism for degradation of the amino acids would presumably be attacked by symmetrical free radicals generated by the Bremsstrahlung irradiation of the solvent. It is difficult to see how such aqueous solution photochemistry could support any asymmetric bias, and the data of Table V are accordingly perhaps to be expected. In the solid state, however, such a mechanism could not be operative, and one might therefore reasonably anticipate direct asymmetric interaction between the circularly polarized Bremsstrahlung photons and the amino acid substrate. This appears to be borne out in the %-Decomposition column

Table V. Radiolysis of Solutions of D,L-Leucine Salts by Strontium-90 Bremsstrahlung[a]

Sample	Solvent	%-Decomposition	Enantiomeric Composition, %					
			Sample			D,L-Standard		
			D	L	(±)	D	L	(±)
D,L-Leu Na Salt	H_2O[b]	48.6	49.4	50.6	0.2	49.3	50.7	0.2
D,L-Leu HCl Salt	H_2O[c]	34.6	50.2	49.8	0.3	50.3	49.7	0.1

[a] β-Source: 56 KCi ^{90}Sr; Dose;4.18×10^8 rads.

[b] With 5% excess NaOH; conc. ~ 1 M.

[c] With 5% excess HCl; conc. ~ 1 M.

Table VI. Radiolysis of Solid D-, L- and D,L-Leucine Samples by Strontium-90 Bremsstrahlung[a]

No.	Sample	% Decomposition	Enantiomeric Composition, %						
			Sample			D,L-Standard			Difference
			D	L	(±)	D	L	(±)	$D-D_{st}$
1	D	11.4							
2	L	16.7							
3	D,L	13.8	50.8[b]	49.2[b]	0.05	50.1[c]	49.9[c]	0.21	0.7
4	D,L	13.8	50.6[d]	49.4[d]	0.25	49.8[d]	50.2[d]	0.29	0.8
		(14.0)[e]	(51.5)[e]	(48.5)[e]					

[a] β-Source: 56 KCi ^{90}Sr; Dose; 4.18×10^8 rads

[b] Average of 3 G.C. Analyses

[c] Average of 4 G.C. Analyses

[d] Average of 5 G.C. Analyses

[e] Calculated from % Decomposition in Nos. 1 and 2.

of Table VI, where we see that the L-leucine has undergone the greatest amount of radiolysis, the D-leucine least, and the D,L-leucine an intermediate amount. From the % Decomposition of D- and L-leucine in Table VI one can calculate that the irradiated D,L-leucine should be about 14.0% decomposed and should have an enantiomeric composition of about 51.5% D- and 48.5% L-leucine. Actual gas chromatographic measurements of the %-Decomposition (13.8%) and enantiomeric composition (50.7% D-, 49.3% L-; average values in Table VI) of the irradiated D,L-leucine gave values close to those calculated. These data thus appear to substantiate the %-Decomposition data, and again to suggest that L-leucine in the solid state undergoes ^{90}Sr radiolysis slightly faster than does D-leucine. Unfortunately, however, the differences in the Enantiomeric Composition (Table VI) between the irradiated sample and the D,L-leucine standard are so close (in our experience) to the experimental error of our gas chromatographic quantitative analytical technique (Bonner, 1972), that we doubt whether a definitive conclusion as to the validity of the Enantiomeric Composition data is possible at the present time. While the %-Decomposition data in Table VI are presumably reasonably accurate (Bonner, 1973), it should be noted that the relatively small differences observed between the D-, L- and D,L-samples could arise alternatively as a result of radiation field inhomogeneities in the ^{90}Sr source employed during the 1.34 year irradiation period. Thus, in themselves, the %-Decomposition data in Table VI can unfortunately not be considered definitive either. Such ambiguities might hopefully be resolved in the future by longer irradiation times. At present we have a similar set of samples which have now been irradiated in the same ^{90}Sr source for a period of some 2.4 years. We propose to examine these samples by similar techniques after a total 3.5-4 year exposure time, whereafter the inconclusive results above can perhaps be clarified, and the question of the origin of molecular chirality by parity violation in β-decay can perhaps be answered definitively.

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